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(54) **ENHANCING HEALTH IN MAMMALS USING TELOMERASE REVERSE TRANSCRIPTASE GENE THERAPY**

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§ 371 (c)(1),
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PCT Pub. Date: **Jul. 3, 2014**

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Related U.S. Application Data

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(51) **Int. Cl.**

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C12N 9/12	(2006.01)
C12N 7/00	(2006.01)
A61K 38/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 9/1276*** (2013.01); ***A61K 48/00*** (2013.01); ***A61K 48/005*** (2013.01); ***C12N 7/00*** (2013.01); *A61K 38/00* (2013.01); *C12N 2710/10341* (2013.01); *C12N 2740/15041* (2013.01); *C12Y 207/07049* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating an age-related disorder in a subject are provided. Aspects of the methods include administering to the subject a nucleic acid vector including a coding sequence for telomerase reverse transcriptase (TERT) and/or telomerase RNA (TR). Gene therapy methods are also provided. Aspects of the invention further include compositions, e.g., nucleic acid vectors and kits, etc., that find use in methods of the invention.

19 Claims, 7 Drawing Sheets

FIG. 1

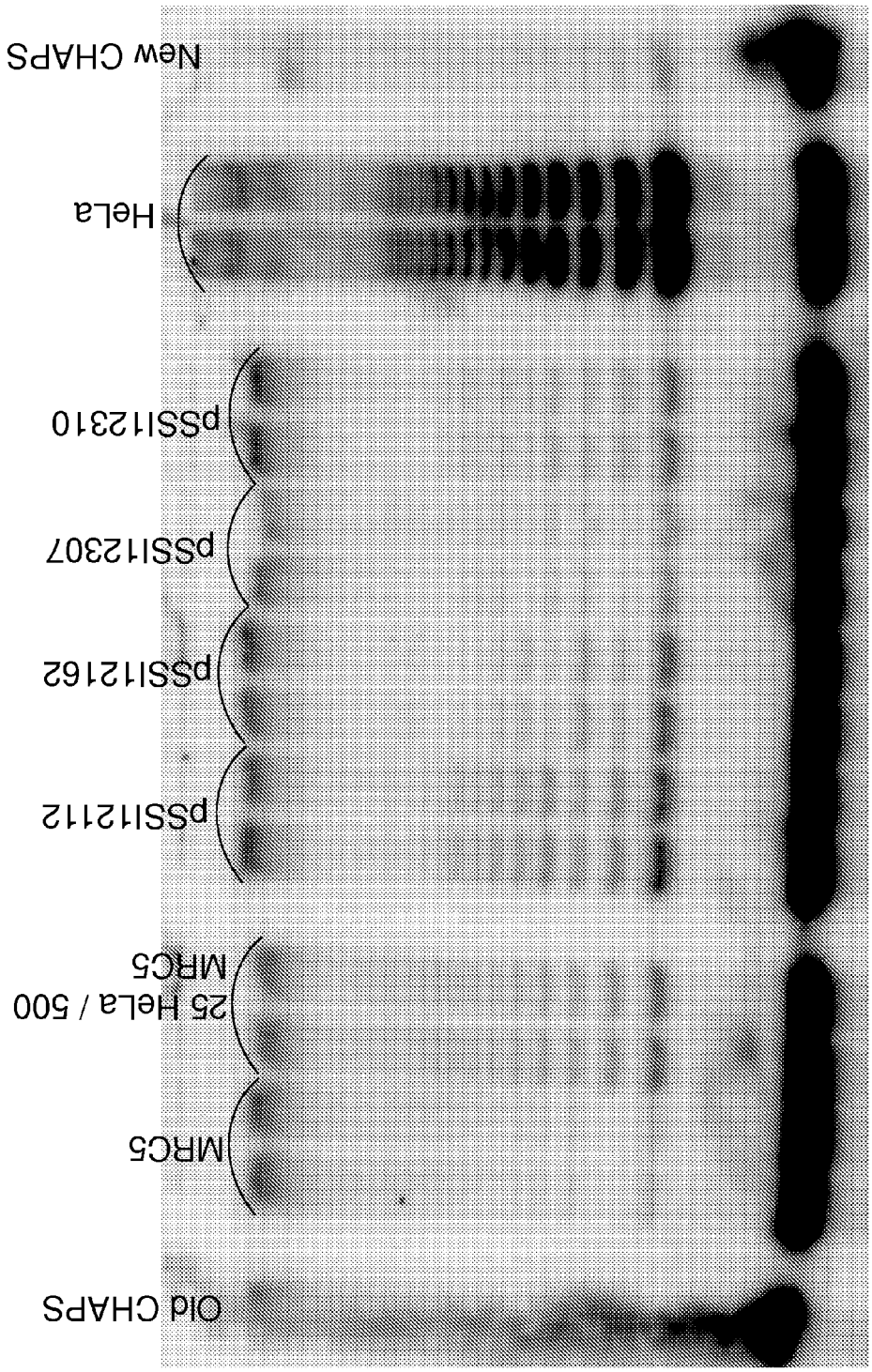

Old CHAPS
MRC5
25 HeLa / 500
MRC5
pSSI12112
pSSI12162
pSSI12307
pSSI12310
HeLa
New CHAPS

FIG. 2

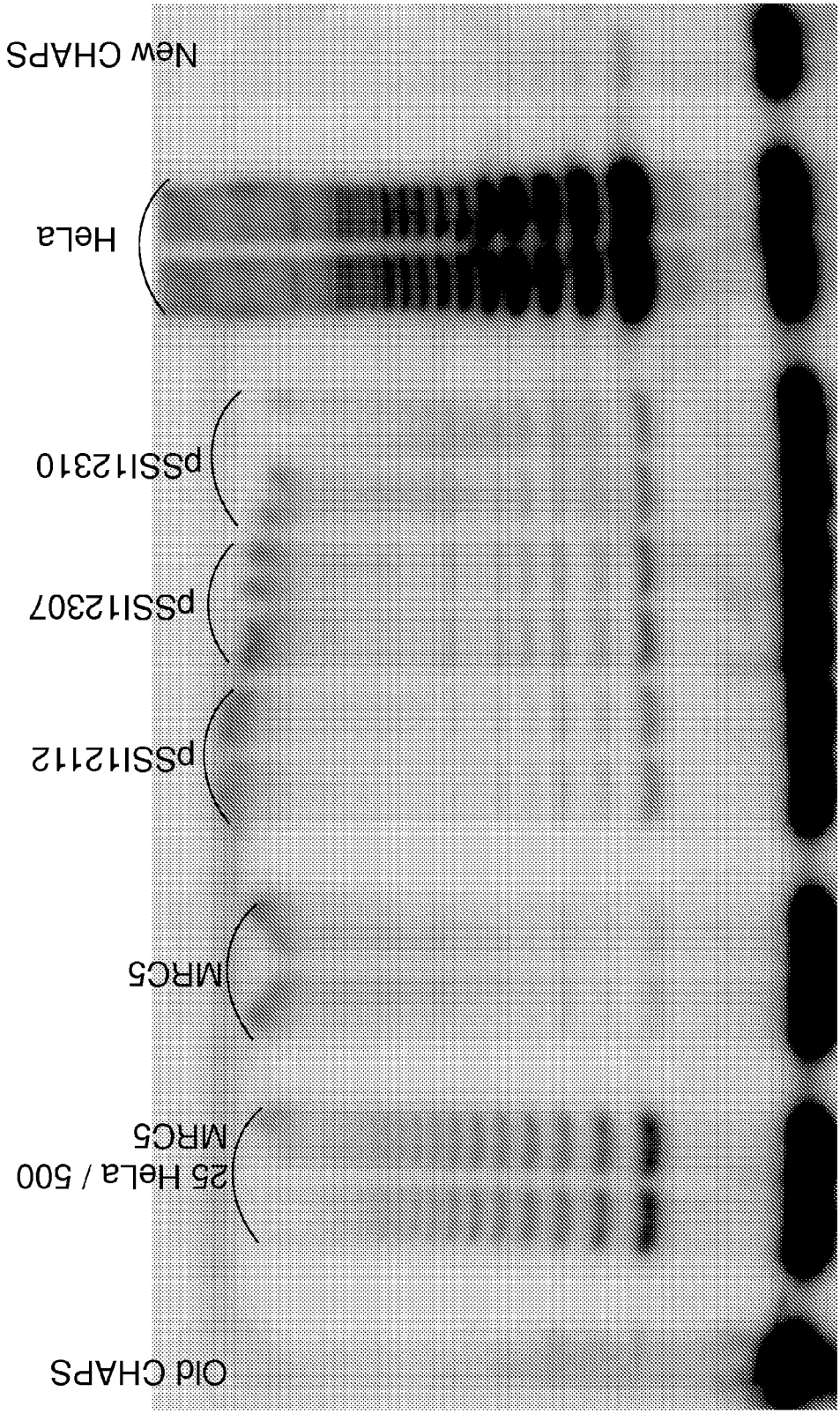

Old CHAPS
25 HeLa / 500 MRC5
MRC5
pSSI12112
pSSI12307
pSSI12310
HeLa
New CHAPS

FIG. 3

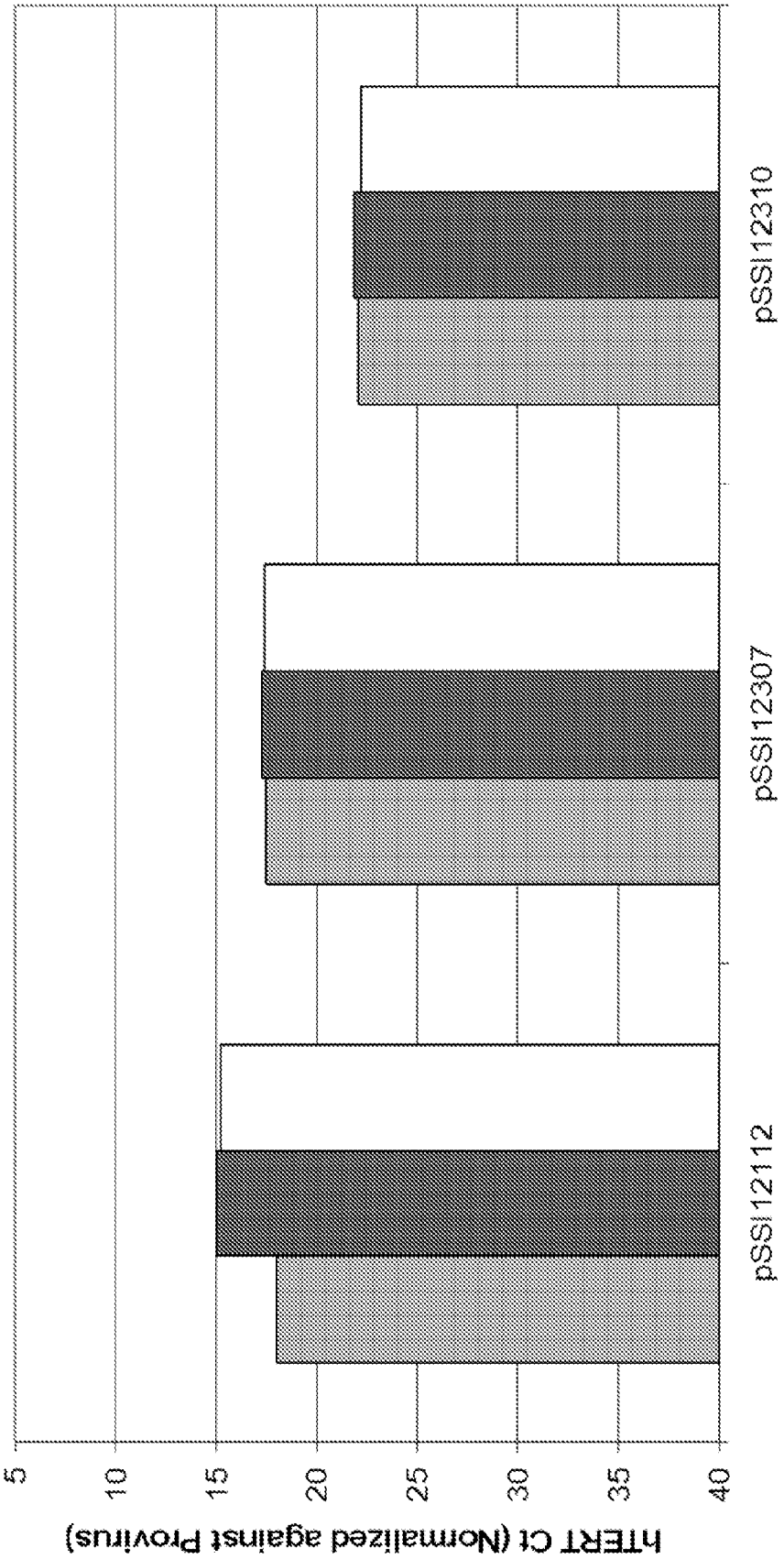

hTERT Ct (Normalized against Provirus)
5
10
15
20
25
30
35
40
pSSI12112
pSSI12307
pSSI12310

FIG. 4

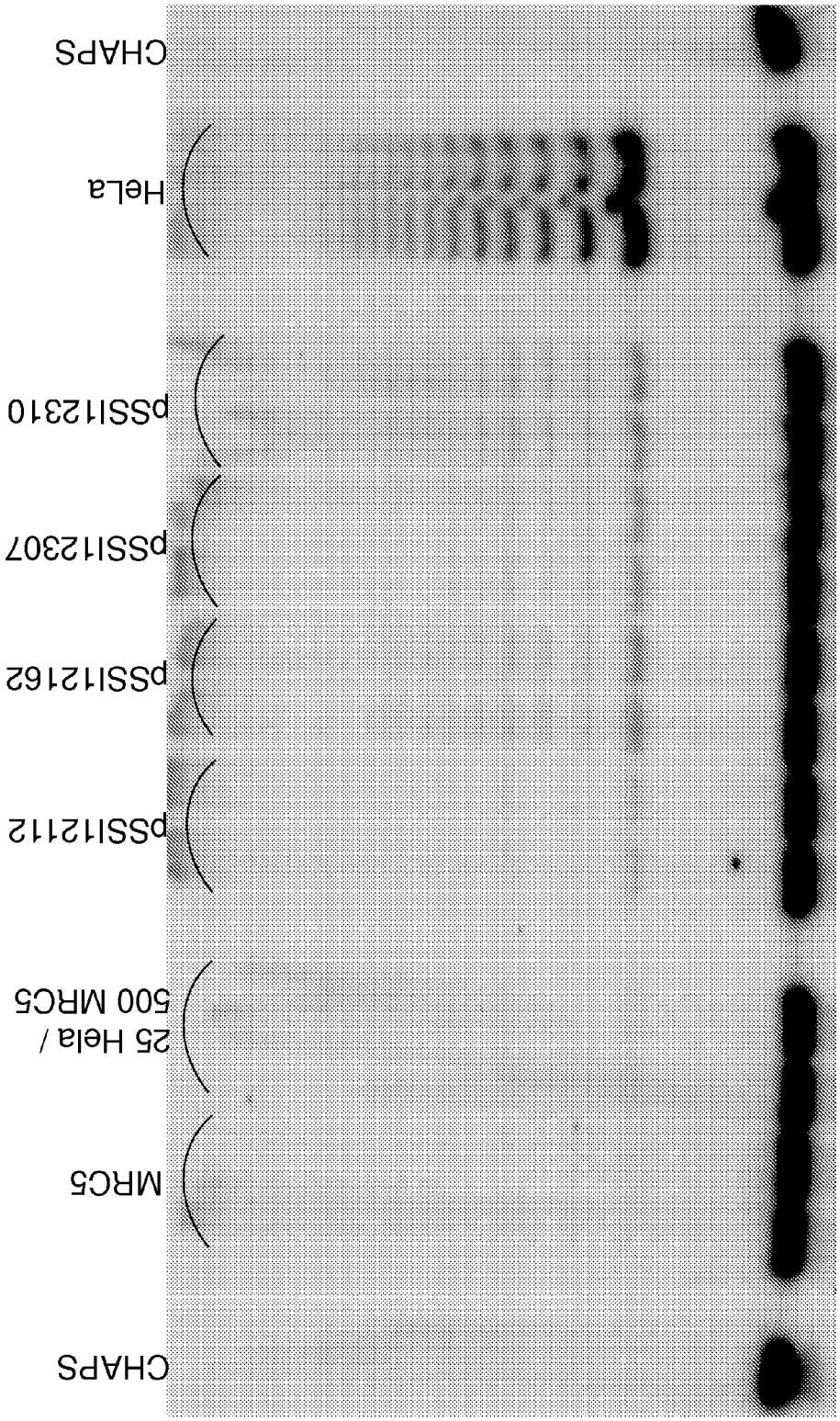

CHAPS
MRC5
25 Hela /
500 MRC5
pSSI12112
pSSI12162
pSSI12307
pSSI12310
HeLa
CHAPS

FIG. 5

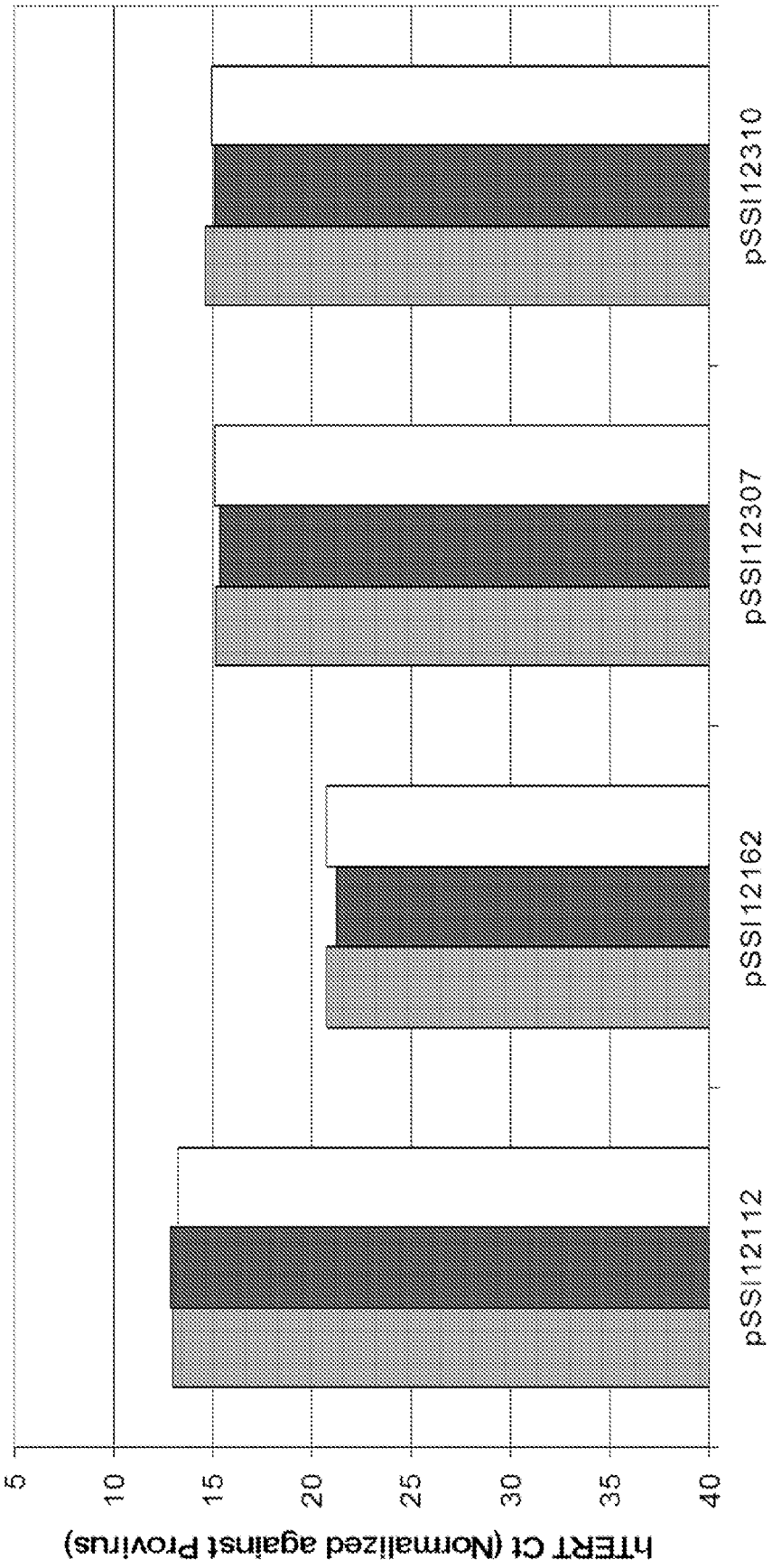

hTERT Ct (Normalized against Provirus)
5
10
15
20
25
30
35
40
pSSI12112
pSSI12162
pSSI12307
pSSI12310

FIG. 6

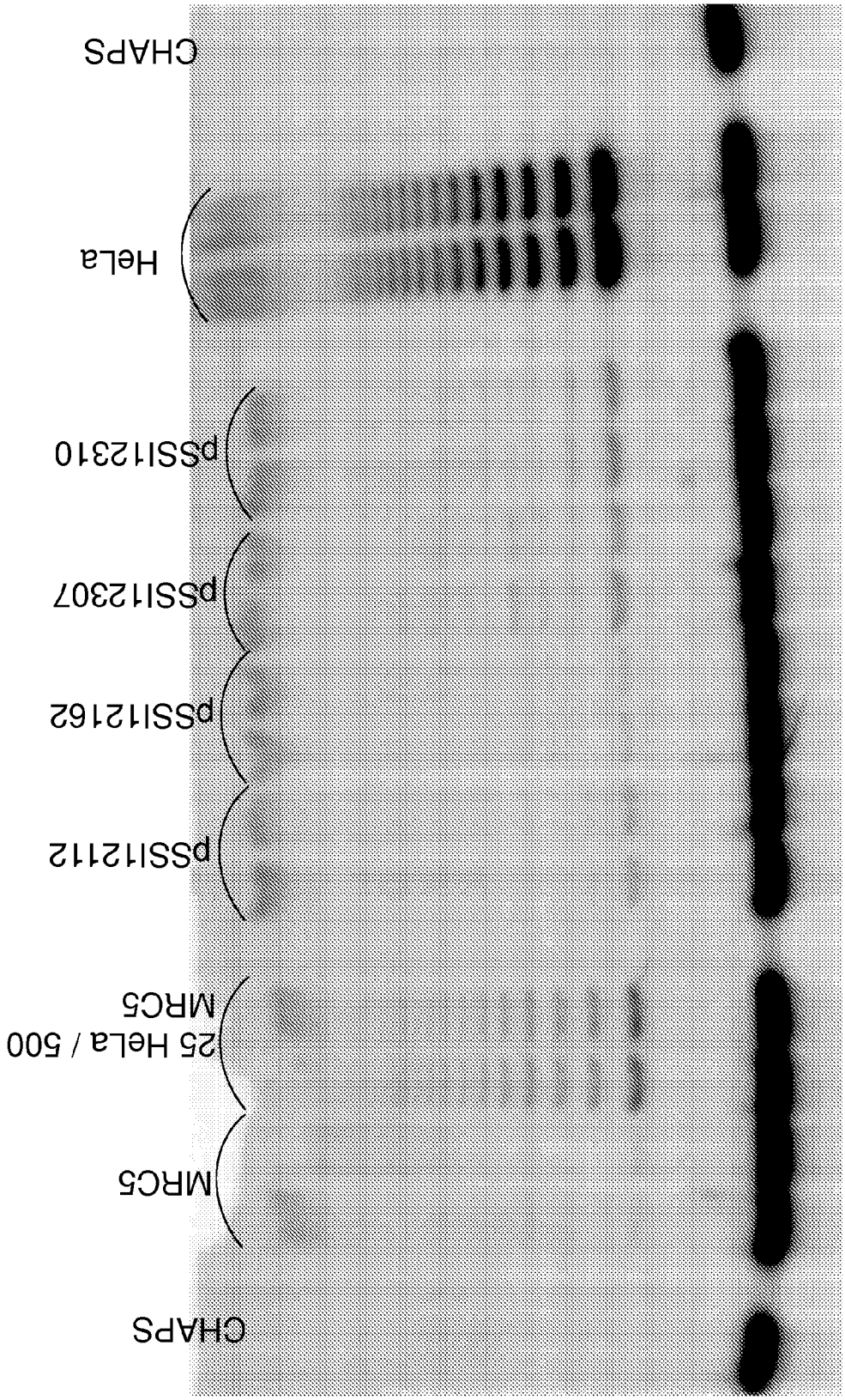
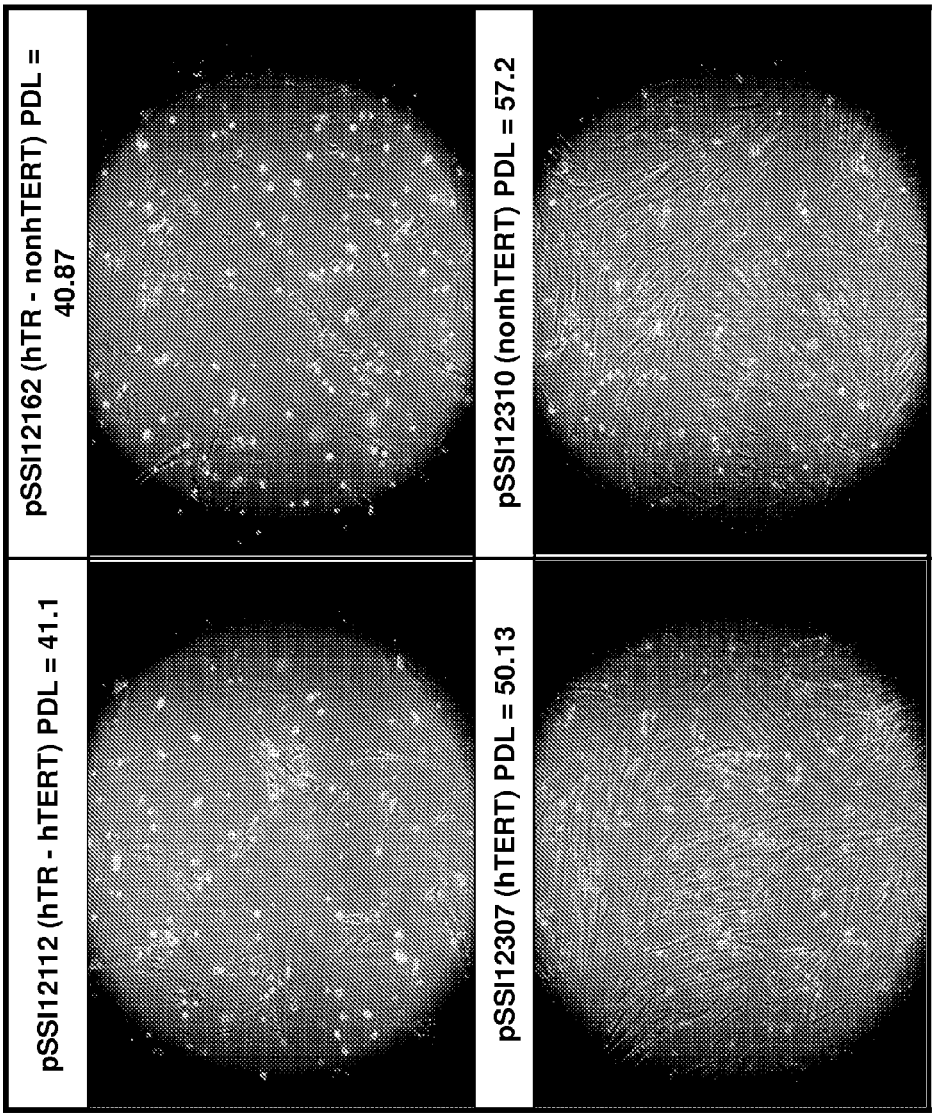

CHAPS
MRC5
25 HeLa / 500
MRC5
pSSI12112
pSSI12162
pSSI12307
pSSI12310
HeLa
CHAPS

FIG. 7


pSSI12112 (hTR - hTERT) PDL = 41.1
pSSI12162 (hTR - nonhTERT) PDL = 40.87
pSSI12307 (hTERT) PDL = 50.13
pSSI12310 (nonhTERT) PDL = 57.2

ENHANCING HEALTH IN MAMMALS USING TELOMERASE REVERSE TRANSCRIPTASE GENE THERAPY

The improvement of health during aging is of interest in aging research. Markers of aging include conditions such as epithelial barrier fitness, osteoporosis, glucose intolerance with insulin insensitivity, loss of memory, and neuromuscular degeneration associated with loss of neuromuscular coordination. For example, bone loss is a well-characterized sign of the aging progress both in mammals including humans which results from bone resorption due to osteoblast insufficiency. Therefore, methods that increase life span and ameliorate various age-related parameters are of interest.

Telomeres are regions of repetitive DNA found at the ends of the chromosomes of most eukaryotes. For example, human telomeres include many kilobases of (TTAGGG)n and are associated with various proteins. Small portions of these terminal sequences of telomeric DNA are lost from the tips of the chromosomes during the S phase of the cell cycle because of incomplete DNA replication. Many human cells progressively lose terminal sequences with cell division, a loss that correlates with the apparent absence of telomerase in these cells. The resulting telomere shortening limits cellular lifespan.

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA. Telomerase is made up of two components: (1) an essential structural RNA component (TR or TER) (in humans the component is referred to as hTR or hTER), and (2) a catalytic protein (telomerase reverse transcriptase or TERT) (in humans the component is referred to as hTERT). Telomerase works by adding multiple DNA sequence repeats to the 2+ end of DNA in the telomere region, where hTER serves as the template for nucleotide incorporation, and TERT as the catalyst. Both the catalytic protein component and the RNA template component of telomerase are activity-limiting components.

SUMMARY

Methods of treating an age-related disorder or condition in a subject are provided. Aspects of the methods include administering to the subject a nucleic acid vector including a coding sequence for telomerase reverse transcriptase (TERT) and/or telomerase RNA (TR). Gene therapy methods are also provided. Aspects of the invention further include compositions, e.g., nucleic acid vectors and kits, etc., that find use in methods of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

FIG. **1** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 7 days post BSD selection, 17 days post infection.

FIG. **2** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **3** illustrates RT-PCR results for hTERT Ct (normalized against provirus) using the vectors pSSI12112, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **4** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 21 days post BSD selection, 31 days post infection.

FIG. **5** illustrates RT-PCR results for hTERT Ct (normalized against provirus) using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **6** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 49 days post BSD selection, 59 days post infection.

FIG. **7** depicts images comparing cell growth of cells that were maintained in 1.5 ug/ml BSD for 55 days post selection using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310.

DETAILED DESCRIPTION

As summarized above, aspects of the invention include methods of treating an age-related disorder in a subject. Aspects of the methods include administering to the subject a nucleic acid vector including a coding sequence for telomerase reverse transcriptase (TERT). In some cases, the vector may include a coding sequence for telomerase RNA (TR). Gene therapy methods that utilize the subject vectors are also provided. Embodiments of the invention include compositions, e.g., nucleic acid vectors and kits, etc., that find use in the subject methods.

The subject methods may lead to increase the expression of telomerase reverse transcriptase and/or telomerase RNA when administered to adult mammals. Administration of the vectors to the subject may extend the lifespan of the subject (e.g., average or maximum lifespan), and may ameliorate one or more markers of ageing, including but not limited to epithelial barrier fitness, osteoporosis, glucose intolerance with insulin insensitivity, loss of memory, and neuromuscular degeneration associated with loss of neuromuscular coordination. The effect may be achieved without increasing the incidence of cancer (malignant neoplastic disease), as assessed by the number of spontaneous neoplasms evident among the population treated.

Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention,

subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, representative illustrative methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

Vectors

As summarized above, one aspect of the invention is a nucleic acid vector. Application of the subject vector to a subject, e.g. using any convenient method such as a gene therapy method, may result in expression of one or more coding sequences of interest in cells of the subject, to produce a biologically active product that may modulate a biological activity of the cell. In some cases, the vector is a nucleic acid vector comprising a coding sequence for telomerase reverse transcriptase (TERT). In some cases, the nucleic acid vector comprises a coding sequence for one or more telomerase components, such as TERT and telomerase RNA (TR). In some embodiments, the vector does not include a cancer suppressing sequence.

In some instances, the vector comprises a coding sequence for telomerase reverse transcriptase (TERT) suitable for use in gene therapy. Gene therapy vectors of interest include any kind of particle that comprises a polynucleotide fragment encoding the telomerase reverse transcriptase (TERT) protein, operably linked to a regulatory element such as a promoter, which allows the expression of a functional TERT protein demonstrating telomerase reverse transcriptase activity in the targeted cells. In some cases, TERT is encoded by the nucleic acid sequence as set forth in SEQ ID NO:1 of WO2012001170 or SEQ ID NO:3 of WO2012001170, or is an active fragment or functional equivalent of TERT. In some instances, the vector include a regulatory sequence which is a constitutive promoter such as the cytomegalovirus (CMV) promoter.

The TERT and/or TR sequence used in the gene therapy vector may be derived from the same species as the subject. Any convenient TERT and/or TR sequences, or fragments or functional equivalents thereof, may be utilized in the subject vectors, including sequences from any convenient animal, such as a primate, ungulate, cat, dog, or other domestic pet or domesticated mammal, rabbit, pig, horse, sheep, cow, or a human. For example, gene therapy in humans may be carried out using the human TERT sequence. In some embodiments, the TERT and/or TR sequence is not a murine sequence.

As used herein, "functional equivalent" refers to a nucleic acid molecule that encodes a polypeptide that has TERT activity or a polypeptide that has TERT activity. The functional equivalent may displays 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 100% or more activity compared to a parent TERT sequence. Functional equivalents may be artificial or naturally-occurring. For example, naturally-occurring variants of the TERT sequence in a population fall within the scope of functional equivalent. TERT sequences derived from other species also fall within the scope of the term "functional equivalent", e.g., a murine TERT sequence. In a particular embodiment, the functional equivalent is a nucleic acid with a nucleotide sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identity to the parent sequence. In a further embodiment, the functional equivalent is a polypeptide with an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%identity to a parent sequence. In the case of functional equivalents, sequence identity should be calculated along the entire length of the nucleic acid. Functional equivalents may contain one or more, e.g. 2, 3, 4, 5, 10, 15, 20, 30 or more, nucleotide insertions, deletions and/or substitutions when compared to a parent sequence.

The term "functional equivalent" also encompasses nucleic acid sequences that encode a TERT polypeptide with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% sequence identity to the parent amino acid sequence, but that show little homology to the parent nucleic acid sequence because of the degeneracy of the genetic code.

As used herein, the term "active fragment" refers to a nucleic acid molecule that encodes a polypeptide that has TERT activity or polypeptide that has TERT activity, but which is a fragment of the nucleic acid as set forth in the parent polynucleotide sequence or the amino acid sequence as set forth in parent polypeptide sequence. An active fragment may be of any size provided that TERT activity is retained. A fragment will have at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 100% identity to the parent sequence along the length of the alignment between the shorter fragment and longer parent sequence.

Fusion proteins including these fragments can be comprised in the nucleic acid vectors needed to carry out the invention. For example, an additional 5, 10, 20, 30, 40, 50 or even 100 amino acid residues from the polypeptide sequence, or from a homologous sequence, may be included at either or both the C terminal and/or N terminus without prejudicing the ability of the polypeptide fragment to fold correctly and exhibit biological activity. Sequence identity may be calculated by any one of the various methods in the art, including for example BLAST (Altschul S F, Gish W, Miller W, Myers E W, Lipman D J (1990). "Basic local alignment search tool". J Mol Biol 215 (3): 403-410) and PASTA (Lipman, D J; Pearson, W R (1985). "Rapid and sensitive protein similarity searches". Science 227 (4693): 1435-41; http://fasta.bioch.virginia.edu/fasta www2/fasta list2.shtml) and variations on these alignment programs.

The vector may further include one or more regulatory sequences. Any convenient regulatory sequences or promoter sequences may be utilized in the subject vectors, e.g., as described herein. In some embodiments, the regulatory sequence that is operatively linked to the coding sequence (e.g., the TERT and/or TR sequence) is the cytomegalovirus promoter (CMV), although any other convenient regulatory sequences may be utilized.

Viral Vectors

Any convenient viruses may be utilized in delivering the vector of interest to the subject. Viruses of interest include, but are not limited to a retrovirus, an adenovirus, an adeno-associated virus (AAV), a herpes simplex virus and a lentivirus. Viral gene therapy vectors are well known in the art, see e.g., Heilbronn & Weger (2010) Handb Exp Pharmacal. 197:143-70. Vectors of interest include integrative and non-integrative vectors such as those based on retroviruses, adenoviruses (AdV), adeno-associated viruses (AAV), lentiviruses, pox viruses, alphaviruses, and herpes viruses.

In some cases, non-integrative viral vectors, such as AAV, may be utilized. In one aspect, non-integrative vectors do not cause any permanent genetic modification. The vectors may be targeted to adult tissues to avoid having the subjects under the effect of constitutive telomerase expression from early stages of development. In some instances, non-integrative vectors effectively incorporate a safety mechanism to avoid over-proliferation of TERT expressing cells. The cells may lose the vector (and, as a consequence, the telomerase expression) if they start proliferating quickly.

Non-integrative vectors of interest include those based on adenoviruses (AdV) such as gutless adenoviruses, adeno-associated viruses (AAV), integrase deficient lentiviruses, pox viruses, alphaviruses, and herpes viruses. In certain embodiments, the non-integrative vector used in the invention is an adeno-associated virus-based non-integrative vector, similar to natural adeno-associated virus particles. Examples of adena-associated virus-based non integrative vectors include vectors based on any AAV serotype, i.e. AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVIO, AAVII and pseudotyped AAV. Vectors of interest include those capable of transducing a broad range of tissues at high efficiency, with poor immunogenicity and an excellent safety profile. In some cases, the vectors transduce post-mitotic cells and can sustain long-term gene expression (up to several years) both in small and large animal models of age-related disorders.

Methods

As summarized above, aspects of the invention include methods of administering a nucleic acid vector to a subject. As such, aspects of the invention include contacting the subject with a viral vector, e.g., as described above, under conditions by which expression of one or more telomerase components (such as TERT and/or TR) in the subject results in a beneficial effect on one or more aspects of the subject's health, including increased longevity, delayed osteoporosis, improved epithelial barrier fitness, improved glucose tolerance, improved memory function, and improved neuromuscular coordination. In some cases, the subject did not develop increased incidence of cancer, illustrating the safety of this type of strategy.

In gene therapy methods, genes are directly inserted into cells affected by an age-related condition so that the function of the cells is normalized by expressing the inserted genes. The gene therapy methods may be used to prevent various diseases or age-related conditions or to reinforce treatment by inserting a specific gene into a body cell and granting a new function to the body cell. One aspect in the treatment of such conditions using gene therapy is that the inserted gene be successfully delivered to the nucleus of the target cell and that the gene be expressed strongly. The gene enters the target cell through endocytosis and is transported into the nucleus to be expressed. The gene can be inserted using a carrier such as a liposome since most DNAs are destroyed when entering the cell. However, most of the liposomes are also destroyed when entering the nucleus, thereby decreasing the transporting efficiency. A virus capable of infecting a human can be treated using gene therapy because the virus effectively inserts exogeneous genes into the human body. Specifically, the gene can effectively be transported and expressed by inserting the gene for the gene therapy into the DNA of the virus using gene recombination and infecting the subject (e.g., a human) with the recombinant virus, which can be mass produced in vitro. In some cases, an adenovirus can be effectively used for the gene therapy by using a mechanism of transporting the gene into the nucleus of the target cell with a high efficiency. In addition, retroviruses are being used in many internationally permissible clinical trials (Wiley—The Journal of Gene Medicine Website: http://www.wiley.co.uk/genetherapy). Retroviruses are effective for gene therapy when inserted into cell chromosomal DNA to allow long term expression of the desired protein.

In certain instances, the expression of the TERT and/or TR following gene therapy according to the invention persists for a time of one or more weeks, such as one or more months, e.g., several months to several years.

When treating specific age related disorders, it is advantageous to target the treatment to the effected tissues. The serotype of the capsid protein of the gene therapy vector may thus be selected based on the desired site of gene therapy, e.g., skeletal muscle tissue for treating neuromuscular coordination.

Any convenient methods may be employed. Methods and vectors of interest that may be adapted for use in the subject invention include, but are not limited to the methods and vectors of WO 2012/001170 and Vidale et al. "The catalytic and the RNA subunits of human telomerase are required to immortalize equid primary fibroblasts." Chromosoma. 2012 Jul. 14. Epub, the disclosures of which are herein incorporated by reference.

In some embodiments, the method of treatment is a gene therapy method and/or the nucleic acid vector used is a gene therapy vector. Gene therapy methods and vectors are well known in the art and generally include delivering a nucleic acid encoding a therapeutically active protein to a subject. The nucleic acid may be delivered in a number of ways including delivering naked DNA such as plasmid or minicircles, the use of liposomes or cationic polymers or other

engineered nano-particles containing the nucleic acid, or viral vectors that encapsidate the nucleic acid.

In a further embodiment, the gene therapy is achieved using stable transformation of organisms with an inducible expression system. In certain embodiments, this aspect of the invention does not extend to human subjects. Expression of TERT or TR can be induced at a later date following transformation, for example, once the subject is an adult or an aged adult, or begins to show signs of age-related disorders. Suitable inducible expression systems are known in the art and include the CRE-LOX recombinase based system and the tetracycline-regulated system.

In some embodiments, the present invention is limited to the expression of TERT an/or TR in adult or aged subjects. In certain embodiments, the methods and vectors are utilized with post-mitotic cells within the subjects, and avoid any increased incidence of cancer.

Any convenient subjects may be treated according to the subject methods. The subject may be an adult animal, such as an adult mammal. The mammal may be a primate, ungulate, cat, dog, domestic pet or domesticated mammal. In some cases, the mammal may be a rabbit, pig, horse, sheep, cow, cat or dog, or a human. In certain embodiments the subject is not a murine mammal. An adult subject treated according to the invention may be aged. The term "aged" is applied to an individual who is older than the period of life during which the individuals of its species are generally healthy and free of chronic illness. According to the present application, an "adult" should be a fully developed individual who has attained reproductive ability, is fertile, or who evidences secondary sex characteristics. As used herein, the term adult when applied to humans, for example, describes early adulthood commencing at around 20 years of age and extending to 39; middle adulthood (40 to 59) and late adulthood (60+). As a comparison, a one year old mouse can be considered to be approximately equivalent in age to a 45 year old human. A 2 year old mouse can be considered to be approximately equivalent to an 80 year old human.

The particular protocol that is employed may vary. Administration of the vectors may be achieved using any convenient protocol. Vectors as described above (e.g., retroviral vectors and lentiviral vectors) may be administered in vivo to subjects by any convenient route. The term "administration" refers to the route of introduction of a formulated vector into the body. For example, administration may be intravenous, intramuscular, topical, oral, or by gene gun or hypospray instrumentation. Thus, administration can be direct to a target tissue or through systemic delivery. Administration directly to the target tissue can involve needle injection, hypospray, electroporation, or the gene gun. See, e.g., WO 93/18759, hereby incorporated by reference herein. Alternatively, vectors of the invention can be administered ex vivo or in vitro to cells or tissues using any convenient transfection techniques.

The vectors of the invention can also be transduced into host cells, including but not limited to, embryonic stem cells, somatic stem cells, or progenitor cells. Examples of progenitor host cells which can be transduced by the vectors of the invention include precursors of erythrocytes and hematopoietic stem cells. In another embodiment, the host cell is an erythrocyte. Transduced host cells can be used as a method of achieving erythroid-specific expression of the gene of interest in the treatment of hemoglobinopathies.

In some embodiments, the method does not include concomitant use of a cancer suppressor.

The step of facilitating the production of infectious viral particles in the cells may be carried out using conventional techniques, such as standard cell culture growth techniques. If desired by the skilled artisan, lentiviral stock solutions may be prepared using the vectors and methods of the present invention. Methods of preparing viral stock solutions are known in the art and are illustrated by, e.g., Y. Soneoka et al. (1995) Nucl. Acids Res. 23:628-633, and N. R. Landau et al. (1992) J. Virol. 66:5110-5113. In a method of producing a stock solution in the present invention, lentiviral-permissive cells (referred to herein as producer cells) are transfected with the vector system of the present invention. The cells are then grown under suitable cell culture conditions, and the lentiviral particles collected from either the cells themselves or from the cell media as described above. Suitable producer cell lines include, but are not limited to, the human embryonic kidney cell line 293, the equine dermis cell line NBL-6, and the canine fetal thymus cell line Cf2TH.

The step of collecting the infectious virus particles also can be carried out using conventional techniques. For example, the infectious particles can be collected by cell lysis, or collection of the supernatant of the cell culture, as is known in the art. Optionally, the collected virus particles may be purified if desired. Suitable purification techniques are well known to those skilled in the art.

Other methods relating to the use of viral vectors in gene therapy can be found in, e.g., Kay, M. A. (1997) Chest 111(6 Supp.): 138S-142S; Ferry, N. and Heard, J. M. (1998) Hum. Gene Ther. 9:1975-81; Shiratory, Y. et al. (1999) Liver 19:265-74; Oka, K. et al. (2000) Curr. Opin. Lipidol. 11:179-86; Thule, P. M. and Liu, J. M. (2000) Gene Ther. 7:1744-52; Yang, N. S. (1992) Crit. Rev. Biotechnol. 12:335-56; Alt, M. (1995) J. Hepatol. 23:746-58;

Brody, S. L. and Crystal, R. G. (1994) Ann. N.Y. Acad. Sci. 716:90-101; Strayer, D. S. (1999) Expert Opin. Investig. Drugs 8:2159-2172; Smith-Arica, J. R. and Bartlett, J. S. (2001) Curr. Cardiol. Rep. 3:43-49; and Lee, H. C. et al. (2000) Nature 408:483-8.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Preferably, the carrier is suitable for administration directly into an affected joint. The carrier can be suitable for intravenous, intraperitoneal or intramuscular administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Another aspect of the invention pertains to pharmaceutical compositions of the vectors of the invention. In one embodiment, the composition includes a vector in a therapeutically effective amount sufficient to treat or prevent (e.g. ameliorate one or more age-related conditions), and a pharmaceutically acceptable carrier. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as treatment or amelioration of an age-related condition. A therapeutically effective amount of vector may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the viral

vector to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the viral vector are outweighed by the therapeutically beneficial effects. The potential toxicity of the viral vectors of the invention can be assayed using cell-based assays or art recognized animal models and a therapeutically effective modulator can be selected which does not exhibit significant toxicity. In a preferred embodiment, a therapeutically effective amount of a viral vector is sufficient to treat or ameliorate one or more age-related conditions in as subject.

Sterile injectable solutions can be prepared by incorporating viral vector in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

It is to be noted that dosage values may vary with the severity of the condition to be ameliorated. It is to be further understood that for any particular subject, specific dosage regimens can be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

The amount of viral vector in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

In Vitro Methods

Also included are in vitro methods, where the subject vectors, e.g., as described above are contacted with a sample. The particular protocol that is employed may vary, e.g., depending on the sample. For in vitro protocols, contact of the vector with the sample may be achieved using any convenient protocol. In some instances, the sample includes cells that are maintained in a suitable culture medium, and the vector is introduced into the culture medium. Depending upon the nature of the vector (e.g., a viral vector), the response desired, the manner of contacting or administration, the number of cells present, various protocols may be employed. The term "sample" as used herein relates to a material or mixture of materials, typically, although not necessarily, in fluid form, containing one or more components of interest.

Utility

The vectors and methods of the invention, e.g., as described above, find use in a variety of applications. Applications of interest include, but are not limited to: research applications and therapeutic applications. Methods of the invention find use in a variety of different applications including any convenient application where the subject is experiencing one or more age-related conditions. In some cases, age-related disorders or conditions that may be modulated or ameliorated using the subject vectors and methods include, but are not limited to, osteoporosis, arthrosis, glucose intolerance, insulin resistant, reduced heart, circulatory and/or lung function, cardiovascular disease, loss of memory, loss of neuromuscular coordination and decrease of longevity, or combinations thereof.

The subject vectors and methods find use in a variety of research applications. The subject vectors and methods may be used to analyze the role of telomerase various biological processes including age-related disorders and conditions.

The subject vectors and methods find use in a variety of therapeutic applications. Therapeutic applications of interest include those applications in which the subject is suffering from one or more age-related disorders or conditions. As such, the subject vectors find use in the treatment of a variety of different age-related conditions in various subjects, and may lead to an extended lifespan. For example, the subject vectors and methods may find use in regulated gene therapy.

Extended lifespan may be an increase in the maximum lifespan possible for any particular species of subject. Extended lifespan may be an increase in the average lifespan of an individual of that species who reaches adulthood. Thus, extended lifespan may be a 5%, 10%, 15%, 20% or more increase in maximum lifespan and/or a 5%, 10%, 15%, 20% or more increase in average lifespan.

The application of the invention extends the period of time for which an individual is generally healthy and free of chronic illness and/or the invention ameliorates disorders that appear often in aged and ageing adult population, including reduced epithelial barrier fitness, osteoporosis, glucose intolerance and neuromuscular degeneration associated with loss of neuromuscular coordination. These are well established indicators of ageing progression.

Accordingly, the invention has beneficial effects in at least one of the following group: reducing the incidence of cancer, on delaying and/or ameliorating osteoporosis, improving epithelial barrier fitness, improving glucose tolerance, improving memory function, and improving neuromuscular coordination. The amelioration of age-related disorders provided by the invention can be as a result of reduction of symptoms in an affected subject or a reduction of incidence of the disease or disorder in a population as compared to an untreated population. The application of gene therapy according to the invention has the effect of treating and/or preventing various age-related conditions and diseases, as assessed by particular markers and disorders of ageing. In a further aspect, therefore, the invention refers to a gene therapy method or the used of a nucleic acid vector as described above, for use in the treatment or prevention in a subject of at least a disorder or marker of ageing that is selected from the group of reduced epithelial barrier fitness, osteoporosis, arthrosis, glucose intolerance, insulin resistanance, loss of memory, loss of neuromuscular coordination, increase in cardiovascular disease, decrease in heart,

circulatory or lung function and decrease in longevity, or combinations thereof. The gene therapy ameliorates at least one marker of ageing, selected for example, from the group of reduced epithelial barrier fitness, osteoporosis, arthrosis, glucose intolerance, insulin resistance, cardiovascular disease, reduced heart and circulatory function, reduced lung function, loss of memory, loss of neuromuscular coordination or decrease of longevity or combinations thereof.

Kits

Aspects of the invention further include kits, where the kits include one or more components employed in methods of the invention, e.g., vectors, as described herein. In some embodiments, the subject kit includes a vector (as described herein), and one or more components selected from a promoter, a virus, a cell, and a buffer. Any of the components described herein may be provided in the kits, e.g., cells, constructs (e.g., vectors) encoding for TERT and/or TR, components suitable for use in expression systems (e.g., cells, cloning vectors, multiple cloning sites (MSC), bi-directional promoters, an internal ribosome entry site (IRES), etc.), etc. A variety of components suitable for use in making and using constructs, cloning vectors and expression systems may find use in the subject kits. Kits may also include tubes, buffers, etc., and instructions for use. The various reagent components of the kits may be present in separate containers, or some or all of them may be pre-combined into a reagent mixture in a single container, as desired.

In addition to the above components, the subject kits may further include (in certain embodiments) instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another form of these instructions is a computer readable medium, e.g., diskette, compact disk (CD), Hard Drive etc., on which the information has been recorded. Yet another form of these instructions that may be present is a website address which may be used via the internet to access the information at a removed site.

Aspects of the invention include providing a virus particle that includes a nucleic acid vector, e.g., as described above. Any convenient virus particles may be utilized, and include viral vector particles described above.

Aspects of the invention include providing a cell that includes a nucleic acid vector. The cell that is provided with the vector of interest may vary depending on the specific application being performed. Target cells of interest include eukaryotic cells, e.g., animal cells, where specific types of animal cells include, but are not limited to: insect, worm or mammalian cells. Various mammalian cells may be used, including, by way of example, equine, bovine, ovine, canine, feline, murine, non- human primate and human cells. Among the various species, various types of cells may be used, such as hematopoietic, neural, glial, mesenchymal, cutaneous, mucosal, stromal, muscle (including smooth muscle cells), spleen, reticulo-endothelial, epithelial, endothelial, hepatic, kidney, gastrointestinal, pulmonary, fibroblast, and other cell types. Hematopoietic cells of interest include any of the nucleated cells which may be involved with the erythroid, lymphoid or myelomonocytic lineages, as well as myoblasts and fibroblasts. Also of interest are stem and progenitor cells, such as hematopoietic, neural, stromal, muscle, hepatic, pulmonary, gastrointestinal and mesenchymal stem cells, such as ES cells, epi-ES cells and induced pluripotent stem cells (iPS cells).

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXPERIMENTAL

Construction of Vectors

pSSI14342: Adeno vector containing hTR and hTERT was constructed. LITR-U1-hTR-CMV-hTERT-SV40pA-RITR Region base locations:

Adeno RITR: 2928-3030
U1 promoter: 6459-6668
hTR: 6850-7300
U1-3' box: 7458-7472
CMV promoter: 7485-8073
Kozak: 8082-8098
TERT: 8087-11482
SV40pA: 11558-11679
Adeno LITR: 5973-6075

pSSI10902: Lentiviral vector pSSI10902 was constructed and contains hTERT, Puro gene (for selection of infected cells) and AmCyan gene (a fluorescent protein for color). In pSSI10902, hTERT is expressed using the CMV promoter, Puro gene is expressed using the SV40 promoter, and AmCyan gene is expressed using the CMV promoter. Below is shown the schematic for the pSSI10902 expression cassette. The sequence of this entire vector is also provided (SEQ ID NO:2).

pSSI10902: 5'-LTR-CMV-hTERT-SV40-Puro-CMV-AmCyan-LTR-2'

Region base locations:

5' LTR: 230-410
CMV promoter: 1883-2374
Kozak: 2627-2643
hTERT: 2632-6027
SV40 promoter: 6053-6286
CMV promoter: 7242-7830
AmCyan: 7891-8577
3' LTR: 9315-9495

pSSI12112: The lentiviral vector pSSI12112 was constructed as a dual vector containing both hTR and hTERT in the same vector. hTR is expressed using the U1 promoter and hTERT is expressed using the CMV promoter. Note: this plasmid also contains the BSD gene being expressed by the PGK promoter which allows selection for cells infected with the lentivirus created using this plasmid. Below is shown a schematic of the expression cassette for pSSI12112. The sequence of this entire vector is also attached (SEQ ID NO:3).

pSSI12112: 5'-LTR-U1-hTR-PGK-BSD-CMV-hTERT-LTR-3'

Region base locations:

5' LTR: 230-410
U1 promoter: 1876-2085
hTR: 2267-2717

U1-3' box: 2875-2889
PGK promoter: 2916-3421
BSD: 3499-3894
CMV promoter: 4023-4611
Kozak: 4620-4636
hTERT: 4625-8020
3' LTR: 8200-8380

Further vectors were constructed and tested as described herein.

pSSI12112=LTR-U1-hTR-PGK-BSD-CMV-TSS-hTERT-LTR

pSSI12162=LTR-U1-hTR-PGK-BSD-CMV-TSS-nonh-TERT-LTR

pSSI12307=LTR-PGK-BSD-CMV-TSS-hTERT-LTR

pSSI12310=LTR-PGK-BSD-CMV-TSS-nonhTERT-LTR

The viral vectors are tested in vitro or in vivo using any convenient methods. Methods of interest that are adapted for use in testing the viral vectors described herein include those methods described by WO 2012/001170 and Vidale et al. "The catalytic and the RNA subunits of human telomerase are required to immortalize equid primary fibroblasts." Chromosoma. 2012 Jul. 14. Epub.

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pSSI12112 (hTR+hTERT) was tested in MRC5 cells. At 7 days post BSD selection, the TRAP activity from pSSI12112 is slightly stronger than the other 3 test samples (pSSI12162 (hTR +non-functional hTERT), pSSI12307 (hTERT), and pSSI12310 (non-functional hTERT)). At 14 days post selection, the pSSI12112 TRAP activity is less than the other 3 samples and eventually diminishes to no TRAP signal at 21 days post BSD selection. FIG. **1** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSS112307 and pSSI12310 where sample cells are collected at 7 days post BSD selection, 17 days post infection.

FIG. **2** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **3** illustrates RT-PCR results for hTERT Ct (normalized against provirus) using the vectors pSSI12112, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **4** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 21 days post BSD selection, 31 days post infection.

FIG. **5** illustrates RT-PCR results for hTERT Ct (normalized against provirus) using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 14 days post BSD selection, 24 days post infection.

FIG. **6** depicts the results of a TRAP Assay using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310 where sample cells are collected at 49 days post BSD selection, 59 days post infection.

FIG. **7** depicts images comparing cell growth of cells that were maintained in 1.5 ug/ml BSD for 55 days post selection using the vectors pSSI12112, pSSI12162, pSSI12307 and pSSI12310.

pSSI14342

(SEQ ID NO: 1)

TCACAGAACCCTAGTATTCAACCTGCCACCTCCCTCCCAACACACAGAGTACACAGTCCTTTCTC

CCCGGCTGGCCTTAAAAAGCATCATATCATGGGTAACAGACATATTCTTAGGTGTTATATTCCACA

CGGTTTCCTGTCGAGCCAAACGCTCATCAAGTGATATTAATAAACTCCCCGGGCAGCTCACTTAA

GTTCATGTCGCTGTCCAGCTGCTGAGCCACAGGCTGCTGTCCAACTTGCGGTTGCTTAACGGGC

GGCGAAGGAGAAGTCCACGCCTACATGGGGGGAGAGTCATAATCGTGCATCAGGATAGGGCGG

TGGTGCTGCAGCAGCGCGCGAATAAACTGCTGCCGCCGCCGCTCCGTCCTGCAGGAATACAACA

TGGCAGTGGTCTCCTCAGCGATGATTCGCACCGCCCGCAGCATAAGGCGCTTGTCCTCCGGGCA

CAGCAGCGCACCCTGATCTCACTTAAATCAGCACAGTAACTGCAGCACAGCACCACAATATTGTT

CAAAATCCCACAGTGCAAGGCGCTGTATCCAAAGCTCATGGCGGGGACCACAGAACCCACGTGG

CCATCATACCACAAGCGCAGGTAGATTAAGTGGCGACCCCTCATAAACACGCTGGACATAAACAT

TACCTCTTTTGGCATGTTGTAATTCACCACCTCCCGGTACCATATAAACCTCTGATTAAACATGGC

GCCATCCACCACCATCCTAAACCAGCTGGCCAAAACCTGCCCCGCCGGGNTATACACTGCAGGG

AACCGGGACTTGGACAATGACAAGTGGGAGAGCCCAGGACTCGTAACCATGGATCATCATGCTC

GTCATGATATCAATGTTGGCACAACACAGGCACACGTGCATACACTTCCTCAGGATTACAAGCTC

CTCCCGCGTTAGAACCATATCCCAGGGAACAACCCATTCCTGAATCAGCGTAAATCCCACACTGC

AGGGAAGACCTCGCACGTAACTCACGTTGTGCATTGTCAAAGTGTTACATTCGGGCAGCAGCGG

ATGATCCTCCAGTATGGTAGCGCGGGTTTCTGTCTCAAAAGGAGGTAGACGATCCCTACTGTACG

GAGTGCGCCGAGACAACCGAGATCGTGTTGGTCGTAGTGTCATGCCAAATGGAACGCCGGACGT

AGTCATATTTCCTGAAGCAAAACCAGGTGCGGGCGTGACAAACAGATCTGCGTCTCCGGTCTCG

CCGCTTAGATCGCTCTGTGTAGTAGTTGTAGTATATCCACTCTCTCAAAGCATCCAGGCGCCCCC

TGGCTTCGGGTTCTATGTAAACTCCTTCATGCGCCGCTGCCCTGATAACATCCACCACCGCAGAA

-continued

TAAGCCACACCCAGCCAACCTACACATTCGTTCTGCGAGTCACACACGGGAGGAGCGGGAAGAG

CTGGAAGAACCATGTTTTTTTTTTTTATTCCAAAAGATTATCCAAAACCTCAAAATGAAGATCTATTA

AGTGAACGCGCTCCCCTCCGGTGGCGTGGTCAAACTCTACAGCCAAAGAACAGATAATGGCATT

TGTAAGATGTTGCACAATGGCTTCCAAAAGGCAAACGGCCCTCACGTCCAAGTGGACGTAAAGG

CTAAACCCTTCAGGGTGAATCTCCTCTATAAACATTCCAGCACCTTCAACCATGCCCAAATAATTC

TCATCTCGCCACCTTCTCAATATATCTCTAAGCAAATCCCGAATATTTAAGTCCGGGCCATTGTAA

AAAATTTGGCTCCAGAGCGCCCTCCACCTTCAGCCTCAAGCAGCGAATCATGATTGCAAAAATTC

AGGTTCCTCACAGACCTGTATAAGATTCAAAAGCGGAACATTAACAAAAATACCGCGATCCCGTA

GGTCCCTTCGCAGGGCCAGCTGAACATAATCGTGCAGGTCTGCACGGACCAGCGCGGCCACTT

CCCCGCCAGGAACCATGACAAAAGAACCCACACTGATTATGACACGCATACTCGGAGCTATGCTA

ACCAGCGTAGCCCCGATGTAAGCTTGTTGCATGGGCGGCGATATAAAATGCAAGGTGCTGCTCA

AAAAATCAGGCAAAGCCTCGCGCAAAAAAGAAAGCACATCGTAGTCATGCTCATGCAGATAAAGG

CAGGTAAGCTCCGGAACCACCACAGAAAAAGACACCATTTTTCTCTCAAACATGTCTGCGGGTTT

CTGCATAAACACAAAATAAAATAACAAAAAAACATTTAAACATTAGAAGCCTGTCTTACAACAGGAA

AAACAACCCTTATAAGCATAAGACGGACTACGGCCATGCCGGCGTGACCGTAAAAAAACTGGTCA

CCGTGATTAAAAAGCACCACCGACAGCTCCTCGGTCAGTCCGGAGTCATAATGTAAGACTCGGTA

AACACATCAGGTTGATTCACATCGGTCAGTGTTAAAAAGCGACCGAAATAGCCNGGGGGAATACA

ATACCCGCAGGCGTAGAGACAACATTACAGCCCCCATAGGAGGTATAACAAAATTAATAGGAGAG

AAAAACACATAAACACCTGAAAAACCCTCCTGCCTAGGCAAAATAGCACCCTCCCGCTCCAGAAC

AACATACAGCGCTTCCACAGCGGCAGCCATAACAGTCAGCCTTACCAGTAAAAAAGAAAACCTAT

TAAAAAAACACCACTCGACACGGCACCAGCTCAATCAGTCACAGTGTAAAAAAGGGCCAAGTGCA

GAGCGAGTATATATAGGACTAAAAAATGACGGTAACGGTTAAAGTCCACAAAAAACACCCAGAAA

ACCGCACGCGAACCTACGCCCAGAAACGAAAGCCAAAAAACCCACAACTTCCTCAAATCGTCACT

TCCGTTTTCCCACGTTACGTCACTTCCCATTTTAAGAAAACTACAATTCCCAACACATACAAGTTAC

TCCGCCCTAAAACCTACGTCACCCGCCCCGTTCCCACGCCCCGCGCCACGTCACAAACTCCACC

CCCTCATTATCATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATGTTAATTAACATGCAT

GGATCCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTC

TTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT

CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG

CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT

CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGA

CTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCC

GCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCT

GTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT

TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC

TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTA

CAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT

CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGC

TGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAG

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CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATAC

GGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTC

CAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTT

ATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATA

GTTTGCGCAACGTTGTTGCCATTGCTGCAGCCATGAGATTATCAAAAAGGATCTTCACCTAGATC

CTTTTCACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGG

CTATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATG

GCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCC

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GGCGCAGGGGATCAAGCTCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGA

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CAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTT

TTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTG

GCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA

CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAG

AAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATT

CGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGAT

CAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAG

GCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCA

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TCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGC

TTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGA

CGAGTTCTTCTGAATTTTGTTAAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAAT

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ACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGC

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AGTGACGTTTTTGGTGTGCGCCGGTGTACACAGGAAGTGACAATTTTCGCGCGGTTTTAGGCGG

ATGTTGTAGTAAATTTGGGCGTAACCGAGTAAGATTTGGCCATTTTCGCGGGAAAACTGAATAAG

AGGAAGTGAAATCTGAATAATTTTGTGTTACTCATAGCGCGTAATACTGGTACCGCGGCCGCCTC

GAGTCTAGAGATATCGAATTCAAGCTTAAGGTGCACGGCCCACGTGGCCACTAGTAATTTTTCTG

CAGAAAACGTACCCGGGGATCCTCTAGGATCCCACCGAAAGGTTGCTCCTTAACACAGGCTAAG

GACCAGCTTCTTTGGGAGAGAACAGACGCAGGGGCGGGAGGGAAAAAGGGAGAGGCAGACGTC

ACTTCCCCTTGGCGGCTCTGGCAGCAGATTGGTCGGTTGAGTGGCAGAAAGGCAGACGGGGAC

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AGGACCGCGGATCGGAAGTGAGAATCCCAGCTGTGTGTCAGGGCTGGAAAGGGCTCGGGAGTG

CGCGGGGCAAGTGACCGTGTGTGTAAAGAGTGAGGCGTATGAGGCTGTGTCGGGGCAGAGCCC

GAAGATCCGGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGCCATTTTTTGTCTAACCCTAAC

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future,

i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

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What is claimed is:

1. A method of treating an age-related disorder in a mammalian subject, the method comprising administering to the subject a nucleic acid viral vector comprising a coding sequence for telomerase reverse transcriptase (TERT) and a coding sequence for telomerase RNA TR to express functional telomerase in the subject.

2. The method of claim **1**, wherein the coding sequence for TERT is human TERT, or an active fragment or functional equivalent of human TERT having at least 80% or more sequence identity to human TERT.

3. The method of claim **1**, wherein the nucleic acid sequence encoding TERT is operably linked to a regulatory sequence that drives expression of the coding sequence.

4. The method of claim **3**, wherein the vector is a non-integrative vector.

5. The method of claim **3**, wherein the vector is an adeno-associated virus-based vector.

6. The method of claim **3**, wherein the vector is a lentivirus-based vector.

7. The method of claim **1**, wherein the vector is represented by one of the following formulae:

LITR-U1-TR-CMV-TERT-SV40pA-RITR

LTR-U1-TR-PGK-BSD-CMV-TERT-LTR

or an active fragment or functional equivalent thereof.

8. The method of claim **7**, wherein the vector is selected from pSSI14342, and pSSI12112.

9. The method of claim **1**, wherein the subject is healthy and free of chronic illness.

10. The method of claim **9**, wherein the subject is an aged adult mammal.

11. The method of claim **9**, wherein the subject is human.

12. The method of claim **9**, wherein the subject is an animal selected from cat, dog and horse.

13. The method of claim **1**, wherein the age-related disorder is selected from the group consisting of osteoporosis, arthrosis, glucose intolerance, insulin resistant, reduced heart, circulatory and/or lung function, cardiovascular disease, loss of memory, loss of neuromuscular coordination and decrease of longevity, or combinations thereof.

14. The method of claim **13**, wherein the method elicits an increase in lifespan in the subject.

15. A method of expressing a functional TERT protein in a mammalian cell, the method comprising:

contacting the cell with a lentivirus-based nucleic acid vector comprising a coding sequence for telomerase reverse transcriptase (TERT) and a coding sequence for telomerase RNA (TR);

to express functional TERT in the cell without toxicity to the cell.

16. The method of claim **15**, wherein the vector is represented by the formula: LTR-U1-TR-PGK-BSD-CMV-TERT-LTR; or an active fragment or functional equivalent thereof.

17. The method of claim **16**, wherein the vector is pSSI12112.

18. The method of claim **15**, wherein the cell is human.

19. The method of claim **15**, wherein the cell is an animal cell selected from cat, dog and horse.

* * * * *